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Green synthesis of gold nanoparticles using palm oil mill effluent (POME): A low-cost and eco-friendly viable approach

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A B S T R A C T

The present study reports the synthesis of gold nanoparticles (AuNps) from gold precursor using palm oil mill effluent (POME) without adding external surfactant, capping agent or template. The biosynthesized AuNps were characterized by using UV–vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). According to the image analysis performed on a representative TEM micrograph by counting 258 particles, the obtained AuNps are predominantly spherical with an average size of 18.75 ± 5.96 nm. In addition, some triangular and hexagonal nanoparticles were also observed. The influence of various reaction parameters such as reaction pH, concentration of gold precursor and interaction time to the morphology and size of biosynthesized AuNps was investigated. This study shows the feasibility of using agro waste material for the biosynthesis of AuNps which is potentially more scalable and economic due to its lower cost.

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1. Introduction

Gold nanoparticles (AuNps) are found to be useful in many applications such as biomedicine, catalysis, biosensing, electronic and magnetic devices. Although existing chemical and physical methods have successfully produced well-defined nanoparticles, these processes are usually costly and involve the use of toxic chemicals. In addition, synthesis of AuNps using chemical methods could still lead to the presence of some toxic chemical species being adsorbed on the surface of nanoparticles which may cause adverse effects in medical applications. In this case, synthesis of nanoparticles using microorganisms or plants could be advantageous, in which biomolecules secreted by the biomass can act as both reducing and capping agents during the reaction and resulted in nanoparticles that are more biocompatible (Lukman et al., 2011).

Over the past several years, biological synthesis of nanoparticles by using microorganisms such as bacteria (Taheri Otaqsara, 2011), fungi (Pradhan et al., 2011), and yeast (Sathish Kumar et al., 2011) have been reported by several research groups. However, a major drawback of microbial synthesis is the difficulty to provide good control over size distribution, shape and crystallinity of nanoparticles (Narayanan and Sakthivel, 2010). The manipulation of reaction parameters such as pH and temperature might inactivate the microbes and hinder the bioreduction process. In addition, the requirements of specialized facilities and long incubation time could limit the scale of production as well. In contrast, plant-mediated synthesis of nanoparticles is comparatively simpler and more cost-effective, in which nanoparticles with morphology comparable to those synthesized by chemical and physical methods were obtained (Parsons et al., 2007).

To the best of our knowledge, the use of agro waste from lignocellulosic plant biomass has not been investigated so far for their ability in biosynthesis of nanoparticles. POME is a residue generated during the production of palm oil. Approximately 0.5–0.75 ton of POME was generated from every ton of oil palm fresh fruit produced and discharged in soil and natural waters as waste (Alam et al., 2009; Chou et al., 2010). In the present study, we have demonstrated the ability of POME to synthesize AuNps without addition of any external surfactant, capping agent or template. The biosynthesized AuNps were found to be predominantly spherical, with some triangular and hexagonal shapes observed. The influence of various reaction parameters to the morphology and size of biosynthesized AuNps was also investigated.

2. Methods

POME was collected from a palm oil mill in Kluang, Johor, Malaysia. It was dried at 60 °C for 24 h before it was ground by an electrical blender and subsequently sieved. The portion of ground POME with diameter of particles ⩽53 μm was used. Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) was procured from Sigma–Aldrich and used as received.

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2.1. Synthesis of AuNps

The source of gold precursor used in all the experiments was $HAuCl_4{\cdot}3H_2O$ in deionized water. Typical reaction mixtures contained 0.1 g of POME powder in 10 ml of 1.0 mM $HAuCl_4$ solution unless otherwise stated. The reaction mixtures were stirred at 300 rpm for 48 h at room temperature. At the end of reaction, the mixture was drawn and filtered for analysis. The effect of pH on the formation of AuNps was investigated by adjusting the pH of reaction mixtures (0.1 g POME, 1.0 mM $HAuCl_4$ solution) from 2.0 to 11.0 by using 0.1–1.0 M HCl and NaOH. Subsequent tests were performed at the optimum pH as determined. The effect of initial gold salt concentration was determined by changing the concentration of $HAuCl_4$ to 0.10, 0.25, 0.50, 1.0 and 2.0 mM. To study the effect of temperature on the formation of AuNps, reaction mixtures containing 0.1 g POME powder, and 10 ml of 1.0 mM $HAuCl_4$ were incubated at 25, 40 and 60 °C, respectively.

2.2. Characterization of AuNps

AuNps were characterized by using a Shimadzu UV2450 UV–vis Spectrophotometer operated at a resolution of 1 nm by using deionized water as blank for each of the sample sets. XRD diffractogram of dry nanoparticles powder was obtained using Bruker-AXS Smart Apex CCD single-crystal diffractometer with CuK_α radiation ($\lambda = 0.1542$ nm). The measurement was carried out using thoroughly dried thin films of nanoparticles on Si(1 1 1) wafers. The morphology of the AuNps was analyzed using high resolution images obtained with a JEOL 3010 TEM.

2.3. FTIR analysis

Samples of POME powder before and after reaction with $HAuCl_4$ as well as the AuNps produced were analyzed. The FTIR spectra were obtained on a Shimadzu IR Prestige-21 operated in transmission mode (400–4000 cm^{-1}) at a resolution of 4.0 cm^{-1} with the sample as KBr pellets.

2.4. Synthesis of AuNps using POME extracts

POME powder (0.1 g) was suspended in 2 ml of extraction solvent, sonicated for 2 h and centrifuged at 14,000 rpm for 10 min at room temperature using the corresponding solvent. The supernatant solutions were collected and kept for future use. During the biosynthesis, the extracted fractions were added to appropriate amount of $HAuCl_4$ and made up to a final volume of 10 ml to obtain a concentration of 1 mM solution. The reaction mixture was stirred for 48 h at room temperature. For the extraction using boiling water, 1 g of the POME powder was suspended in 50 ml of water and the mixture was boiled for 10 min.

3. Results and discussion

3.1. Effects of initial pH on the biosynthesis of AuNps

The initial pH value of the aqueous $HAuCl_4$ solutions was an important parameter in the synthesis of AuNps using POME. It was observed that the color of gold solutions changed from pale yellow to purplish-pink (pH 2.0, 3.0) and wine red (pH 4.0, 5.0) followed by violet and grayish blue (pH 6.0, 7.0, 8.0, 9.0) when the initial pH of solution was increased. However, no color change was observed for gold solutions at highly alkaline pH (pH 10.0 and 11.0). The color change would be an indication of gold bioreduction mediated by POME and the subsequent formation of AuNps after 48 h of reaction. The UV–vis spectra obtained from solutions at different initial pH after 48 h of reaction shows characteristic absorption bands of AuNps at around 530–560 nm except for pH 10.0 and 11.0 (Supplementary data Fig. S1a). The shift of wavelength could be an indication of the increasing aggregation of AuNps occurred at higher pH.

In addition to biosynthesis of AuNps, the capability of POME to bind and concentrate gold from aqueous solutions was also observed at acidic pH. It was found by ICP-MS analysis, the maximum efficiency of gold biosorption reached ~95% at pH 2.0 after 2 h of reaction. However, the solution turned to pink color after 24 h, suggesting the formation of AuNps in solution after long contact time. Similar result was also observed by Antunes et al., where the optimal removal of gold ions was found at pH 2.0 within 3 h of reaction time (Antunes et al., 2001). In general, biosorption mechanism of gold is ionic rather than covalent due to the dependence of gold binding with pH. At pH 2.0, gold is present in solution in anionic form $[AuCl_4]^-$ and the functional groups of active biocompounds on the biomass surface such as hydroxyl groups tend to undergo protonation and become positively charged. The overall positively charged surface could promote the interaction between protonated functional groups and the negatively charged $[AuCl_4]^-$ through electrostatic attraction or electrovalent bond (Zhou et al., 2010). As a result, biosorption was preferred over bioreduction of gold ions. The bioreduction of gold could occur through the oxidation of hydroxyl to carbonyl groups as shown in Eq. (1):

$$AuCl_4^- + 3R{-}OH \rightarrow Au^0 + 3R{=}O + 3H^+ + 4Cl^- \qquad (1)$$

POME has a high content of phenolic acids and flavonoids. The abundant hydroxyl groups available in these compounds could participate in the gold bioreduction. The formation of AuNps at pH 2.0 after 24 h suggested that the bioreduction of gold ions is a slow process at acidic pH due to the protonation of hydroxyl groups and the competing biosorption process.

At higher initial pH values (10.0 and 11.0), the increasing amount of OH^- could act as strong complexing agents of gold ions that interfere with the capping ability of biomolecules in POME and also compete with the $[AuCl_4]^-$ for binding to the biomolecules.

3.2. Effect of the of $HAuCl_4$ concentration and temperature on the biosynthesis of AuNps

According to LaMer model, it is predicted that the formation of nanoparticles could only happen when the precursor concentration is within a suitable range for nucleation. However, this range might vary amongst different biomass-assisted synthesis approaches. Therefore, the effect of precursor concentration on this POME-mediated synthesis was studied (Supplementary data Fig. S1b). It was found that there was no formation of AuNps by using 0.10 and 0.25 mM of gold chloride. The formation of AuNps increased as the concentration of gold chloride increased from 0.50 to 2.0 mM. This is in agreement with the range of initial precursor concentration ($\leqslant 10^{-3}$ M) that was generally used by most of the biological methods reported in the literature (Ghodake et al., 2010; Shankar et al., 2003; Zhou et al., 2010).

The effect of temperature on the formation of AuNps was also investigated (Supplementary data Fig. S1c). The solution changed from pink to grayish blue followed by dark brown as the temperature increased. The darkening of solution indicates that the process of aggregation of smaller AuNps to form larger particles was favored over nucleation to form new nanoparticles at higher temperature. The slight shift of absorption wavelength from 530 to 545 nm when the temperature was increased from 25 to 60 °C suggested the increasing size of AuNps. This phenomenon may due to the increased rate of reduction at higher temperature which may hinder the capping process and resulted in the aggregation of AuNps. In fact, the effect of temperature in directing the shape and size of nanoparticles has been reported by several research groups (Ghodake et al., 2010; Lukman et al., 2011; Njagi et al., 2011). The ability of POME to biosynthesize well-dispersed AuNps at room temperature could not only increase the ease of handling but also provide the advantage of energy-saving.

3.3. Characterization of AuNps

The morphology of AuNps synthesized at pH 3.0 and pH 8.0 was observed by using TEM. The TEM images revealed that AuNps formed at pH 3.0 were predominantly spherical with some others having occasionally triangular, truncated triangular and hexagonal shapes. When the pH was increased to 8.0, the edges of the nanostructures became not well-defined which could be a result of aggregation. It was found that pH 3.0 appears to be an optimum pH for this biosynthesis process due to the well-dispersed and uniform nanostructures obtained. AuNps with well-defined structures were observed after 1 h of reaction which suggested that the biosynthesis process mediated by POME was relatively rapid. As the reaction proceeded for about 9 and 48 h, aggregation of AuNps became dominant with some of the nanoparticles overlapped with each other and clustered together. This observation revealed that reaction time could be an important factor in controlling the size and morphology of AuNps. In fact, the formation of AuNps could be a result of heterogeneous nucleation and growth followed by Ostwald ripening. The heterogeneous nucleation could be catalyzed by extraneous material released by POME, in which the foreign particles act as a scaffold for the crystal to grow on. Since there is no requirement for the incipient surface energy, this nucleation process is kinetically favored. Along with the crystal growth, Ostwald ripening took place and responsible for the coarsening of size distribution. During Ostwald ripening, small crystals tend to dissolve in the solution and transfer their mass to grow larger particles which lead to a greater stability with lower energy state. The excess Gibb's free energy associated with the nanoparticles could then be minimized by transformation into more energetically favorable shapes, which was directed by the bioorganic capping molecules (Fiehn et al., 2000).

Fig. 1 shows the histogram of particle size distribution of the AuNps obtained after 3 h of reaction time at pH 3.0. The average particle size was determined to be 18.75 ± 5.96 nm by counting 258 particles on a representative TEM micrograph. The co-existence of AuNps in smaller and larger size was due to the AuNps formed in early and later stages of the reaction, which shows that both nucleation to form new nanoparticles and aggregation to form larger particles happened consecutively. Crystalline nature of the biosynthesized AuNps was investigated by XRD technique and the corresponding XRD-diffractogram was analyzed. Diffraction peaks observed at 38.1°, 44.1°, 64.7° and 77.8° can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg's reflections of cubic structure of metallic gold respectively (JCPDS No. 04-0784). Another series of diffraction peaks at 45.9°, 57.0°, 71.6° and 76.1° which can be indexed to the (1 0 0), (1 0 3), (0 0 6) and (1 0 5) Bragg's reflections of hexagonal phases (JCPD No. 41-1402) were also observed (data not shown). XRD analysis suggested that the biosynthesized AuNps are composed of crystalline gold and they are biphasic in nature. In addition to the assigned Bragg peaks, additional yet unassigned peaks found at 28.4° and 32.1° were also noticed, which might be a result of crystallization of some bioorganic compounds or proteins that are present in the POME on the surface of AuNps. Similar results were also reported by Shankar et al. (2003) and Philip (2009) in the synthesis of Ag nanoparticles using geranium leaf and mushroom extract.

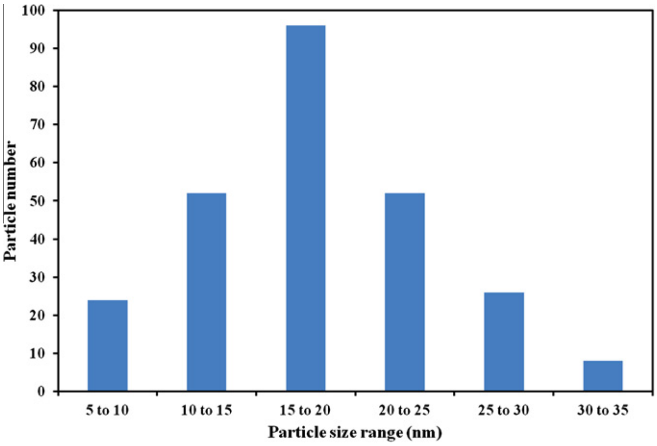

Fig. 1. Histogram of particle size distribution of AuNps synthesized using POME at pH 3 with reaction time of 3 h.

The stability of the AuNps synthesized by POME was examined by comparing the UV–vis spectra of AuNps that were freshly prepared and stored at 4 °C for more than 6 months (Supplementary data Fig. S1d). There is no obvious change of solution color and SPR peaks observed after 6 months of storage which would suggest the AuNps synthesized have considerately good stability.

3.4. Possible functional groups involved in the biosynthesis of POME

The involvement of surface functional groups in biosynthesis of AuNps was studied by FTIR (data not shown). The main difference between the spectrum of POME before and after gold reduction is the disappearance of N–H or C═O stretching vibrations band at 2351 cm^{-1}. This phenomenon revealed that biomolecules in the POME such as proteins or flavonoids that contain abundant of C═O or N–H groups might play an important role in the bioreduction and stabilization of AuNps during the synthetic process. FTIR analysis of AuNps shows the presence of five bands at 1056, 1645, 2376, 2924 and 3453 cm^{-1}. The first two absorption bands are attributed to the C–N stretching of aliphatic amines or amines/phenols and amide I bands, respectively. The other two bands at 2376 and 2924 cm^{-1} are characteristic of N–H or C═O and C–H stretching vibrations. The reappearance of 2376 cm^{-1} in the spectrum of AuNps suggested the attachment of some C═O or N–H groups onto AuNps during the biosynthesis process. In addition, the band at 3453 cm^{-1} indicates the presence of polyphenolic OH group. Hence, it was deduced that AuNps might be capped and stabilized by functional groups on the POME surface such as proteins and polyphenols through the interactions of free amine groups in the proteins as well as the carbonyl groups in the polyphenols.

In order to further investigate the compounds involved in the bioreduction of gold ions, reducing capability of various polarity based fractions of POME extracted using *n*-hexane, ethyl acetate, butanol, unboiled and boiled water were analyzed. Formation of AuNps was found by using both boiled and unboiled water extracts of POME. This observation suggested that most of the bioorganic compounds involved in the synthesis of AuNps are polar and water-soluble, such as flavonoids, proteins, reducing sugars, and water-soluble alkaloids present in plant systems. The minor amount of AuNps formed by using ethyl acetate extract was believed to be mediated by polyphenols and alkaloids extracted by this medium polar solvent. No formation of AuNps was observed by using the butanol extract. In fact, butanol is more polar than ethyl acetate. However, the bioactive polyphenols present in the POME might be more soluble in polar-aprotic solvent such as ethyl acetate rather than polar-protic solvent such as butanol. It was also found that AuNps were not formed by using hexane extract which may due to the inability of this non-polar solvent to extract alkaloids and polyphenols.

3.5. Interaction of biosynthesized AuNps with mercury ions

AuNps could form alloys with mercury with varying composition such as Au_3Hg, AuHg, $AuHg_3$ (Pradeep and Anshup, 2009). The potential use of AuNps for the removal of mercury from water through amalgamation of these two metals have been demonstrated (Lisha et al., 2009). The interaction of these biosynthesized AuNps with mercury was examined, which will correspond to its potential use in water purification. For the treatment of AuNps with mercury, 50 mg of $HgCl_2$ was added into 5 ml of the as prepared AuNps. According to the UV–vis spectra, shift in peak position was observed for AuNps before and after mercury treatment, which suggested that AuNps were consumed to form Au–Hg amalgam with different morphology (data not shown). A rapid color change of AuNps solution from wine red to grayish after the addition of Hg(II) was observed within seconds. The interaction of AuNps with Hg(II) shows the chemical reactivity of the biosynthesized AuNps and its potential use for mercury removal in water purification.

4. Conclusions

Biosynthesis of AuNps using POME was demonstrated to be a simple, low-cost and non-toxic method. The AuNps synthesized were predominantly spherical with an average size of 18.75 nm. The morphology and size of AuNps could be controlled by varying the reaction conditions such as initial pH of the $HAuCl_4$ solution and reaction temperature. Bioactive compounds involved in the biosynthesis are most likely proteins and water soluble polyphenols in POME that contains amine and carbonyl groups. Interaction of biosynthesized AuNps with Hg(II) was investigated which demonstrates the chemical reactivity of these nanoparticles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.01.015.

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